ET-109 August 1937

United States Department of Agriculture
Bureau of Entomology and Plant Quarantine

METHODS FOR THE FIXING AND PRESERVATION, FOR EXHIBIT PURPOSES, OF THE NATURAL GREEN COLOR OF PEA PLANTS AND PODS

By Francis E. Carroll, Division of Truck
Crop and Garden Insect Investigations

The methods herein described have given satisfactory service in the fixing and preservation of the natural green color of samples of pea vines and pods which were prepared in order to demonstrate, for exhibit purposes, the contrast between pea plants which had been treated with rotenone-containing insecticides for the control of the pea aphid and untreated pea plants grown under identical conditions. Two methods were used and for convenience they may be designated as the "copper acetate method" and the "copper chloride method."

Copper Acetate Method

The plants to be processed are cut close to the ground and placed immediately in water to keep them turgid until they are used in the fixing process.

The bath for breaking down the natural green color and fixing the artificial color is made from copper acetate as follows: A convenient volume of 50 percent acid is shaken with an excess of cupric acetate [$Cu(CH_3COO)_2H_2O$] until saturated with that salt, then the supernatant liquid is decanted and diluted with four times its volume of distilled water. For extensive fixing operations it is necessary to prepare several liters of this diluted solution.

In the process of fixing, a sufficient quantity of the diluted solution is placed in a vessel which is large enough to permit complete submergence of the plants in the solution. The solution is then heated until it is brought to a gentle boil. At this time two plants - one plant which has been treated with the insecticide and one untreated plant - are then submerged in the liquid and boiled for 15 to 20 minutes. After the first 3 to 5 minutes of boiling, the green plants become yellow. As boiling continues, an artificial green color replaces the natural color. The fixing process should be continued until the artificial green color satisfactorily matches the natural green color of the pea plants. Small plants usually do not require processing as long as larger ones. The degree

of color of the processing plants should be compared carefully and frequently with other plants selected from the same lot but not being processed. This comparison should be made both for treated and untreated plants because the color of the two kinds of plants may be different at the time they are selected. The fixing process is completed in the pods and tender tips a few minutes before it is completed in the remainder of the plants. Therefore, it is necessary to watch them carefully and remove them from the bath in time. As soon as the proper intensity of color is reached the samples are removed from the bath and immediately washed in lukewarm tap water. Next they are passed through two or three changes of water and then placed in a solution of formaldehyde made by adding 5 parts of commercial formalin to 95 parts of distilled water.

In the process of preserving, the processed plants are placed singly in museum jars of the inverted type. The jars are filled with the solution of formaldehyde. It is necessary to change this solution several times over a period of 2 or 3 weeks until the solution remains clear. When finally the solution remains clear, the museum jars are closed by the following method: The jars are placed in a bath of warm water, the water reaching well up around the neck of the jars. The temperature of the water is gradually increased until it is between 125° and 140° F. The formaldehyde solution in the jars is brought up flush with the constricted opening of the neck. A porous cork is then inserted into the constricted opening. As the entire jar remains in the hot water bath the air is permitted to come up through the top of the cork. This process continues for a period of approximately 1 hour or until there is no further evidence of bubbles inside of the jar. The top of the cork is then dried with a cloth and a stick of picein is melted, by means of a Bunsen burner, to cover the cork. Enough of the material is melted onto the cork so that there is approximately 3/4 inch of picein covering. The water bath is then cooled gradually. This cooling process pulls the cork and picein securely down into the neck of the bottle.

Copper Chloride Method

Although the copper chloride method is not so satisfactory as the copper acetate method, it has the advantage of being simpler in its application than the latter. The copper chloride solution is prepared according to the proportions given in the following formula:

50 percent ethyl alcohol	90.0 cc.
Commercial formalin	5.0 cc.
Glycerine	2.5 cc.
Glacial acetic acid	2.5 cc.
Copper chloride (cupric) ($CuCl_2.2H_2O$)	10.0 grams.
Uranium nitrate	1.5 grams.

These materials are mixed in the sequence given. For extensive fixing operations it is necessary to prepare approximately ten times the quantity of solution given in the formula.

After the materials are dissolved in the liquid, specimens of the plants are submerged in the liquid and left until they reach a green color as close as possible to their natural green before being processed. Small plants reached this color in 2 or 3 days, while larger and more sturdy plants required a longer period - sometimes 7 to 10 days. Plants preserved in this manner make satisfactory dried specimens when pressed and dried properly. Pea pods cut from the plants may be preserved by this method with fair success but the pods do not have the natural green color of the pods preserved by the copper acetate method. The pods cannot be dried in the same manner as the plants, but after being fixed in the copper chloride solution they may be placed in the formaldehyde solution and cleared over a period of several weeks, the same as is necessary with the copper acetate method. It is difficult to determine the proper intensity of green when using this method, owing to the fact that the liquid has such a deep green color. It is necessary, therefore, to remove some of the plants and pods from the liquid at daily intervals to note their color.